Final author comments: **Phytoplankton biomass, composition, and productivity along a temperature and stratification gradient in the Northeast Atlantic Ocean**

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The manuscript will undergo a major revision based on the valuable comments of the reviewers. We thank the reviewers for their work, which has improved the manuscript. In short, a supplement will be provided which states model equations, and group specific P vs E parameters. Furthermore, the supplement provides data on microscopy samples that were analyzed in concert with CHEMTAX analysis. Furthermore, pigment ratios used for CHEMTAX are provided in the supplement.

In addition changes were made to the photoacclimation assumptions for the productivity model. For the previous calculations low light acclimation was assumed at Chl-a concentrations in excess of 0.5 mg m^{-3}. This has been reconsidered and changed. The changes are supported by onboard experiments that investigated the photoacclimation state of the phytoplankton, using recovery of photosynthetic efficiency from excess light exposure as a measure for photoacclimation state. These experiments will be included in the revised ms. Due to these changes, productivity values higher latitudes in summer were higher (18%), whereas values for oligotrophic stations in spring were lower (26%). Graphs and correlations have changed accordingly. The changes had minor effects on the overall conclusions drawn from this work.

Please note that one co-author was added to the manuscript (P.D. Rozema), due to his involvement with the photoacclimation experiments.

Furthermore, hypotheses were included in the introduction.

Detailed enquiries are answered below.

<u>Response to referee #2</u>

1) Model validation: If validation against directly comparable PP (or P vs E) measurements is not possible, then I suggest that a more comprehensive comparison to previous PP measurements is necessary (i.e. extend section 4.3). Are there additional published datasets to include here? What different methods were used? What are the reasons for any differences?
Using CHEMTAX to obtain community structure can be problematic, particularly when applied over large special scales as in the case in this study. Presumably, details are in Mojica et al. (submitted), but I feel more information is needed here as well because the model, and much of the interpretation, is highly dependent on it. In particular, please state how well CHEMTAX performed against validation and ground truthing. PP estimates presented in this manuscript are entirely dependent on results in Mojica et al. (submitted), as such, they cannot fully be evaluated until Mojica et al.is published.

The following section will be included in a supplement.

Comparison of CHEMTAX with light microscopy and flow cytometry

The taxonomic information obtained by CHEMTAX was compared with light microscopy observations on fixed sampled and with flow cytometry data (Mojica et al. submitted). For light microscopy, 100 ml seawater was fixed by 1 ml of Lugol iodine solution, supplemented with 0.5% glutarealdehyde in dark bottles. Based on CHEMTAX, 18 samples (7 and 11 from spring and summer, respectively) were selected for light microscopic analysis and compared with the taxonomic data obtained with CHEMTAX. Fifty ml of fixed sample was concentrated by sedimentation (24 h) and observations were made on an Olympus IMT-2 inverted microscope, using 20 and 40 times magnification for phytoplankton larger and smaller than 20 µm, respectively. The microscopy observations are briefly discussed below.

In summer, the haptophytes *Phaeocystis sp* (free cells, on bladders and in colonies) increased in concentration from low to high latitude (38,000 up to 2,208,000 cells l^{-1}, dominating the phytoplankton biomass at higher latitudes). Diatom concentrations in summer were low and increased from low to higher latitudes (0-2,000 up to 25,000 cells l^{-1}, *Pseudo-Nitzschia delicatessima*, *Nitzschia longissima*). Larger diatoms were found at low concentrations at high latitudes (*Rhizosolenia*, *Proboscia* sp <1,000 cells l^{-1}). Small dinoflagellates (< 15 µm) appeared mostly heterotrophic (concentrations 22,000-100,000 cells l^{-1}). Larger dinoflagellates were observed at higher latitudes in low concentrations (*Ceratium* sp, < 2,000 cells l^{-1}). In spring, *Phaeocystis* was not abundant, but small *Emiliania huxleyi* like cells were abundant at mid-latitudes (7,074,887 cells l^{-1}). However, the presence of this species was not confirmed by flow cytometry. Furthermore, unidentified pico-eukaryotes were abundant (584,000-4,162,433 cells l^{-1}) at low and mid latitudes in spring. Small diatoms (*Chaetoceros* sp and *Nitszchia longissima*) concentrations were around 3,760 cells l^{-1} at stratified stations, whereas small (presumably heterotrophic) dinoflagellates were around 4,000 cells ml^{-1}. Large dinoflagellates (*Ceratium* sp.) were found in concentrations of 40 cells l^{-1}. At non-stratified stations, large diatoms (*Chaetoceros* sp., *Thalassiosira* sp., *Proboscia* sp., *Rhizosolenia* sp., dominated the phytoplankton community at latitude 25 °N. At higher latitudes, large (>20 µm) Prasinophytes (5,600 cells l^{-1}) and Cryptophytes (6,000 cells l^{-1}) were observed, whereas diatom concentrations were lower.

Data obtained by flow cytometry will be presented in detail by Mojica et al. (submitted). Patterns obtained by flow cytometry of *Synechococcus* spp. and *Prochlorococcus* spp. were comparable with those obtained by pigment composition. However, flow cytometry abundance of *Prochlorococcus* spp. in the upper 50 m of oligotrophic stations in summer was higher than the contribution to Chl-a suggested from pigment composition.

Direct comparison of phytoplankton composition between these methods is complicated by the differences in units and by the specific limitations of each method. Flow cytometry provides abundance data of phytoplankton groups that are smaller than 20 µm, including some groups that are difficult to identify using light microscopy (e.g. small eukaryotes, *Prochlorococcus* spp., and *Synechococcus* spp.). Light microscopy gives detailed information on larger phytoplankton species. In contrast, CHEMTAX provides taxonomic information relative to Chl-a for phytoplankton with a size range > 0.7 µm. In this respect, all methods are complementary to each other. Overall, patterns in phytoplankton composition obtained by light microscopy and flow cytometry were in agreement with CHEMTAX. The dominance of phytoplankton with a haptophytes pigment signature, and the low contribution of diatoms in summer to the phytoplankton community were revealed by light microscopy and CHEMTAX. The dominance of diatoms at higher latitudes in spring was observed by both CHEMTAX and light microscopy. Also the overall low contribution of (photosynthetic) dinoflagellates was shown by CHEMTAX and light microscopy. In oligotrophic waters, increasing dominance of

Prochlorococcus spp., *Synechococcus* spp. was shown by CHEMTAX and flow cytometry with decreasing latitude.

2) Please provide more details on the P vs E culture data, and justify their use in the PP model. **A supplement will be prepared where a table is included with the P vs E data previously published by Kulk et al 2011.**
For example, what were the culture conditions?
Culture conditions will be briefly stated in the supplement.

Additional information for primary production calculations

Calculation of irradiance
The irradiance calculation were based Kirk (1994, 2010). Surface irradiance was calculated according by $E\ t\ = E_m \cdot sin\ \ \pi \cdot \frac{t}{N}$
where E_m (mol m^{-2}) is the maximum irradiance, t (h) is time, and *N* is day length (h). Irradiance at depth was calculated using the attenuation coefficient:

$E_{n,t} = E_{n-1,t} \cdot e^{-K_d \cdot dz}$
where *E* (mol m^{-2}) is irradiance, t (h) is time, K_d is the attenuation coefficient (m), and dz (m) is layer thickness.

Primary production calculations
The equation of Platt et al. (1980) was used to calculate the primary production at depth:

$$P = P_S\ \ 1 - e^{-\alpha\ \frac{E}{P_s}}\ \ \ \ e^{-\beta\ \frac{E}{P_s}}\ \ - P_0$$

where *P* is the chlorophyll *a* specific CO_2 fixation rate (µg C µg Chl-a^{-1} h^{-1}) at irradiance *E* (µmol photons m^{-2} s^{-1}), P_S is the theoretical maximum for photosynthesis in the absence of photoinhibition (µg C µg Chl-a^{-1} h^{-1}), α is the initial rate of photosynthesis (µg C µg Chl-a^{-1} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$), β is a measure of photoinhibition (µg C µg Chl-a^{-1} h^{-1} [µmol photons m^{-2} s^{-1}]$^{-1}$), and P_0 was used to indicate respiration or dark carbon fixation at zero irradiance.

Partitioning Chl-a between five taxonomic groups
The bio optical model calculates primary production for five taxonomic phytoplankton groups. The characteristics of these groups were determined from ^{14}C based photosynthesis versus irradiances (PE) measurements of *Prochlorococcus marinus* (group 1), *Synechococcus* sp. (group 2), *Ostreococcus* sp. (group 3), *Emiliania huxleyi* (group 4), and *Thalassiosira oceanica* (group 5). Photosynthetic characteristic of low light (50 µmol photons m^{-2} s^{-1}) and high light (125 µmol photons m^{-2} s^{-1}) acclimated phytoplankton were used (supplement table 1).

The partitioning of chlorophyll-a between the taxonomic groups was based on HPLC pigment analysis and CHEMTAX calculations (see below), resulting in eight different taxonomic groups. The Chl-a of three taxonomic groups was assigned to other phytoplankton groups for the calculation of primary production. Chl-a of dinoflagellates was assigned to the haptophytes (group 4) and Chl-a of cryptophytes and pelagophytes was combined with that of the prasinophytes (group 3).

Relative importance of taxonomic groups

To visualize the importance of the different parameters for the respective taxonomic groups, integrated productivity was calculated for a station assuming 100 % contribution of a single group for high and low light acclimated conditions, respectively (supplement table 2). Productivity was highest for diatoms and lowest for *Prochlorococcus*. Changes in photoacclimation were most important for *Prochlorococcus* and diatoms, i.e. PE parameters for high light acclimation resulted in 55% higher productivity compared with low light acclimation.

Sensitivity of the model to changes in Chl-a, K_d, and photosynthetic parameters
The values of the photosynthetic parameters ($P_{s,,}$ α, β, P_0), Chl-a and K_d were varied by 20% to assess the sensitivity of the production model to changes in photosynthetic parameters, Chl-a, and K_d. The model was most sensitive to changes in Chl-a, a 20% change resulted in a 20% change in productivity. A 20% change in P_s and K_d resulted in a 16% change in productivity. Finally a 20% change in β, and P_0 resulted in 10 and 2% change in productivity, respectively.

Supplement table 1. Photosynthetic parameters used in the production model. The theoretical maximum for photosynthesis in the absence of photoinhibition (P_s in μ μg C μg Chl-a^{-1} h^{-1}), the initial rate of photosynthesis (α in μg C μg Chl-a^{-1} h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$), photoinhibition (β in μg C μg Chl-a^{-1} h^{-1} [μmol photons m^{-2} s^{-1}]$^{-1}$), and respiration or dark carbon fixation at zero irradiance (P_0 in μ μg C μg Chl-a^{-1} h^{-1}) are given for low light (50 μmol photons m^{-2} s^{-1}) and high light (125 μmol photons m^{-2} s^{-1}) acclimated cultures of *Prochlorococcus marinus* eMED4, *Synechococcus* sp. (RCC477 and RCC543), *Ostreococcus* sp. (clade B), *Emiliania huxleyi*, and *Thalassiosira oceanica* are given. Experiments were performed using exponentially growing cultures (12-12 h light-dark cycle) at 20°C. Values represent the mean of two cultures. Data from Kulk et al. (2011).

	Low light acclimated				**High light acclimated**			
	P_s	α	β	P_0	P_s	α	β	P_0
Prochlorococcus marinus	2.17	0.032	0.002	0.036	5.05	0.031	0.002	-.2187
Synechococcus sp.	5.45	0.121	0.003	0.205	4.72	0.062	0.003	0.154
Ostreococcus sp.	7.96	0.097	0.006	0.229	10.13	0.097	0.004	0.424
Emiliania huxleyi	50.83	0.091	0.176	0.398	13.39	0.785	0.008	0.461
Thalassiosira oceanica	18.61	0.071	0.012	0.306	229.2	0.153	0.350	1.461

Supplement table 2. Daily depth integrated productivity (mg C m^{-2} day^{-1}) calculated for a random station assuming 100 % contribution to chlorophyll *a* of one taxonomic phytoplankton group, for low light (LL, 50 μmol photons m^{-2} s^{-1}) and high light (HL, 125 μmol photons m^{-2} s^{-1}) acclimated conditions.

	LL	**HL**
Group 1 Prochlorococcus marinus	479	1064

Group 2 Synechococcus sp.	972	1302
Group 3 Ostreococcus sp.	1544	1912
Group 4 Emiliania huxleyi	1500	1859
Group 5 Thalassiosira oceanica	2200	4904

Are the cultured phytoplankton suitable representatives of the population in the study region? ***Synnechococcus*, and *Prochlorococcus* were observed throughout the cruise, and were routinely enumerated by flow cytometry. Diatoms including *Thalassiosira* species were only observed in spring, whereas other diatom species (mostly Nitzchia sp.) were observed in low numbers in summer, as observed by light microscopic analysis of lugol fixed samples. *Ostreococcus* was not identified although small pico eukaryote cells were abundant in flow cytometry enumerations, and the marker pigment prasinoxanthin was consistently observed. *Emiliania huxleyi* as a representative of the haptophytes was observed in summer and spring but was never dominating the phytoplankton community.**

What are the implications of assuming fixed P vs E parameters for each group? Could the culture conditions bias the modeled PP contribution of different groups in any way? Culture details are presumably in Kulk et al. 2012, but it would be useful to include more detail here because they are a crucial component of the model. **The following will be included in the supplement:**

Relative importance of taxonomic groups
To visualize the importance of the different parameters for the respective taxonomic groups, integrated productivity was calculated for a station assuming 100 % contribution of a single group for high and low light acclimated conditions, respectively (supplement table 2). Productivity was highest for diatoms and lowest for *Prochlorococcus*. Changes in photoacclimation were most important for *Prochlorococcus* and diatoms, i.e. PE parameters for high light acclimation resulted in 55% higher productivity compared with low light acclimation.

Sensitivity of the model to changes in Chl-a, K_d, and photosynthetic parameters
The values of the photosynthetic parameters ($P_{s,}$ α, β, P_0), Chl-a and K_d were varied by 20% to assess the sensitivity of the production model to changes in photosynthetic parameters, Chl-a, and K_d. The model was most sensitive to changes in Chl-a, a 20% change resulted in a 20% change in productivity. A 20% change in P_s and K_d resulted in a 16% change in productivity. Finally a 20% change in β, and P_0 resulted in 10 and 2% change in productivity, respectively.

3) Is it possible to validate, for example, the modeled bulk community P vs E curves against curves measured during this or previous studies in the region? Halsey et al. 2011 is cited to justify assuming no effect of nutrient limitation on the P vs E parameters for each group. Should this reference be Halsey et al. 2010 Photosynth Res 103:125-137? From what I understand, whether or not P vs E curve parameters vary as a result of nutrient availability depends on the method used to obtain the P vs E curve. In particular, the timescale over which the experiments were conducted and whether or not they quantify net or gross (and C or Chl –specific) PP. Please include sufficient information on the P vs E data to reassure the reader that this assumption is appropriate for the current study.

PvsE data were included in the supplement

4) Other specific comments: I would urge the authors to clearly highlight the novel aspects of the work. The main conclusions (Section 5) focus on the statistical relationships between SST, nutrient, Chl-a and PP, but it is not entirely clear how these relationships build on current understanding? It would also be helpful to specify what is learnt from the (novel) group-specific PP estimates? **The introduction and discussion will be revised, providing hypotheses, and more structure.**

5) Throughout the manuscript, chlorophyll-a concentration is assumed to represent phytoplankton biomass. I don't feel this assumption is appropriate or necessary for the current study. The factors that decouple chl-a from biomass (incl. temperature, nutrients and light availability, community composition) are explicitly dealt with in the manuscript. I suggest simply referring to Chl-a concentration throughout i.e. simply change the term "biomass" to "chl-a". (e.g. see Perez et al. 2006 DSR-I 53:1616-1634 for the difference between Chl-a and carbon biomass in the oligotrophic N. Atlantic).
In our opinion Chl-a is still a valid parameter for phytoplankton biomass. Suggested new title: "Phytoplankton Chlorophyll-a biomass, composition and productivity along a temperature and stratification gradient in the Northeastern Atlantic Ocean".

6) Section 2.6.2. It would be helpful to include specific details on the primary production model, including key model equations. **Equations will be included in the supplement (see above).**

7) P1795 L25: "Phytoplankton growth in the oceans depends on seasonal and interannual climatological cycles that determines the availability of nutrients and light." Also mention top-down (grazer) controls.
This was included in the introduction:
Phytoplankton growth in the oceans ultimately depends on seasonal and inter-annual climatological cycles that determine the availability of nutrients and light. In addition, loss factors such a grazing, viral lyses, and the sinking influence phytoplankton standing stock.

8) P1798 L23: "potential (1-125m): : :" What is meant by "potential" euphotic zone? Do you mean "entire"? **The maximum the euphotic zone depth that was observed in this cruise (125 m).**

9) P1800 L15: "Depth integrated chl-a was then calculated for the euphotic zone and for defined depth intervals: : :. total depth-integrated Chl-a (surface to 200-410m)" It would be helpful to state what determines the depth interval for each location (is Chl-a negligible at these depths?) in order to reassure the reader that the variability in the integration depth does not influence the patterns shown in Fig 4. **This will be included:** Chl-a was negligible below 200 and 410 m, respectively.

10) P1799 L4 "We defined oligotrophic stations as those stations where NO3 in the upper euphotic zone was below the detection limit" Please quote the detection limit.
Detection limit 0.03 µmol/l will be included

11) The spectrally weighted mean specific absorption coefficient (a) was calculated as the sum of a*ph between 400-700 nm, and corrected by a normalized solar

spectrum (maximum set to one). " Does this mean that the change in light spectrum with depth was not accounted for in the spectral correction of a*ph?
In these calculations the change in light spectrum with depth was not accounted for. This will be stated in the method section.

12) If so, please make this clear and acknowledge any potential errors resulting from this assumption. **In the discussion it will be stated that spectral changes in light with depth were not accounted for in the model.**

13) P1801 L10: "The current study focused on five phytoplankton groups used in the primary production model". To make a clearer distinction between the groups identified by CHEMTAX and the groups used in the primary production model, consider changing to: "In the current study, five of the eight identified phytoplankton groups were resolved in the primary production model". **This is a misunderstanding, the Chl-a from groups that were not represented in our model species was assigned to one of these groups. This is stated in the method and will also be stated in the supplement.**

Also, does this mean primary production is likely to be underestimated, because not all groups are considered in the primary production model? If so, please give some indication of the magnitude of the underestimation.
No, groups with no P vs E data were assigned to other groups (Chl-a from dinoflagellates were assigned to the haptophyte group. Pelagophytes and cryptophytes were assigned to the prasinophyte group. This is stated in the method.
The model could be expanded with other groups this will be stated in the discussion.

14) P1801 L25: "The daily light dose at each station was obtained using data (level 3, 9 d average) from the : : :. MODIS satellite". Please specify the name of the data product.
Photosynthetically available radiation MAMO_PAR_9km.CR MODIS-Aqua 9 km^{-2} resolution obtained from Giovanni ocean color radiometry portals

15) P1808 L13-L25: "The inverse relationships between SST and near surface phytoplankton biomass and PP0-50m for stratified stations suggests that within the SST range of 13-23oC, North Atlantic open ocean productivity can co-vary with seasonal, inter annual and multi-decadal SST changes. This also implies that anthropogenic warming of the ocean has a negative influence on phytoplankton biomass and productivity in the stratified open ocean within this temperature range. : : :. etc" Take care when using correlations measured along a transect to predict future changes in response to long-term or climate warming. I suggest either removing these kinds of assertions or substantiating them with due consideration of the relevant processes (including the extensive knowledge of these processes in the literature).

This section was revised:
The inverse relationships between SST and near surface phytoplankton biomass and $PP_{0–50m}$ for stratified stations suggests that within the SST range of 13–23 ◦C, North Atlantic open ocean phytoplankton productivity co-varries with SST. If this also applies to seasonal, inter annual, and multi-decadal SST changes, this would imply that anthropogenic warming of the ocean has a negative influence on phytoplankton biomass and productivity in the stratified open ocean within this temperature range. It should be noted that these correlations are not proof of causation. Nevertheless, the existence of correlations between SST, nutrient concentrations, phytoplankton pp and Chl-a in the surface oceans provide support for the

hypothesis that SST influences nutrients in the open ocean surface and thereby controls phytoplankton biomass, productivity and composition.

16) Technical corrections:
Define abbreviations on first use. E.g. P1798 L6: “CTD” P1798 L19: “NOX” P1799 L16: “HPLC” **(NO_2 +NO_3)**
After defining an abbreviation, only use abbreviated terms. E.g. P1799 L14, change “Chlorophyll” to “Chl a”.
Check consistency of abbreviations used. E.g. P1804 L12-14: Check use of abbreviation N and P – should they be NO3 and PO4? Note that the abbreviation “N” is elsewhere used to mean “North”. Also change N to NO3 in Table 1 and caption.

P1806 L25: Should “Fig 7” be Fig 5c? **This should be Fig 5, 7**

P1805 L13: “Oligotrophic stations showed low surface Chl a, whereas higher concentrations were found in the deep chlorophyll maximum”. This is implicit in the term “deep chlorophyll maximum”, so is this sentence necessary?
Sentence was changed: Oligotrophic stations showed a deep chlorophyll maximum, whereas surface Chl-a was lower than that of mesotrophic stations.

Figure 2. Should the figure names be (A,B), (C,D,) and (E,F) instead of (A), (B), (C)? **Legends of figure 2 are changed accordingly.**